Note on the Surface Electric Charges of Living Cells.

By W. B. Hardy, F.R.S., and H. W. Harvey.

The movement of free living cells suspended in a fluid through which an electric current is passing towards one or other of the poles has been described by many observers. In almost every case the movement has been observed in thin films of fluid under a cover-glass mounted in the way usual for microscopical examination. The cells do not always all move in the same direction; some migrate towards the anode, others to the cathode, and Thornton[*] found that in mixed suspensions of diatoms and amœbæ, or yeast cells and red blood corpuscles, the animal cells migrated to the anode, the vegetable cells to the cathode. He infers from this that animal and vegetable cells are oppositely electrified, the former being negative, the latter positive, to the fluid.

It is obvious at the outset that there are exceptions to this generalisation, for Becholt[†] describes a movement of bacteria towards the anode, the direction being reversed after agglutination. Dale[‡] and Lillie[§] also have described movements of animal cells to the cathode, but Thornton points out with some justice that in these cases the cells were not in their normal habitat.

The objection does not, however, apply to a natural culture of Gonium, Vorticella, and Amœba. In thin films such as Thornton used we found that the first two moved towards the cathode, while the amœbæ moved to the anode.

The movement of living cells, or indeed of any suspended particle, in films of liquid a millimetre or less in depth enclosed between glass plates is not open to the simple interpretation which Thornton places upon it. Arising from a contact difference of potential at the glass-water interfaces the upper and lower surface films of the water are dragged along in the electric field with considerable velocity on account of the ions they contain, and the flow along the boundary so produced is compensated under hydrostatic pressure by a return flow in the middle or round the edges of the stratum of water, if it be thick enough. The velocity of a particle past the observer is the sum of the velocity of the fluid and the velocity of the particle *through* the fluid, and

[*] 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 638.

[†] 'Zeit. Phys. Chem.,' 1904, vol. 48, p. 385.

[‡] 'Journ. of Physiol.,' vol. 26, p. 219.

[§] 'Amer. Journ. of Physiol.,' 1893, vol. 8, p. 273.

since water is usually positive to glass and to particles suspended in it, the apparent velocity is commonly the sum of two velocities of opposite sign. Both movements, that of the water and that of the particle, are remarkably dead beat in thin films. If the bodily flux of fluid throughout the whole thickness under one field of the microscope were zero, and the stream lines were constant, the average velocity of particles taken through this entire thickness would, of course, give the mean velocity relative to the fluid.

When a suspension of yeast cells and red corpuscles in isotonic sugar is observed in a U-tube wide enough to reduce the flux of fluid practically to zero, *both* migrate to the anode, but the corpuscles travel much the faster. In Thornton's experiment, therefore, the yeast cells move past the observer towards the cathode because they are unable to stem the current of water which is travelling in that direction. Yeast and blood cells under the conditions of the experiment are not oppositely electrified. Both are negative to the fluid, but the yeast cells migrate more slowly.

When the depth of the fluid is increased to 2·5 mm., the yeast and blood cells are seen to move in the same direction in the middle regions and in opposite directions in the film of fluid next the glass floor of the cell, and also in that next the surface.

Troughs of various shapes were used by us to observe these movements. Good results were got with one 2 cm. long, 3 mm. wide, and 2·5 mm. deep, with parallel glass sides, which opened at each end into a wide portion, divided into two compartments by a porous plate. The outermost compartment at each end was filled with a saturated solution of zinc sulphate, into which dipped electrodes of amalgamated zinc. The fluid was not covered in any way.

The current was between 0·0001 and 0·002 ampère, and was not allowed to run in the same direction for more than a few seconds at a time. With a current of more than 0·01 ampère, the vapour density rose to a point at which it deposited on the front of the objective—a remarkable result, which can be attributed only partly to heating. The image was, as a rule, completely "fogged" by dew when the current had run 4 seconds, the front glass of the objective being about 4 mm. above the surface of the fluid.

The difference in the apparent movement of red corpuscles and yeast cells in the layer next the glass, and in the middle of a stratum of liquid 2·5 or more millimetres deep, is not due to a difference in the interface between the cells and the fluid, for the velocity of one kind of cell with respect to the other was the same in both regions.

Countings of the number of divisions of a micrometer scale which contiguous yeast and blood separated from each other during a run of the

current gave, for the layer next the glass, where they moved in opposite directions, 0·79 division per second, and for the middle region, where they moved in the same direction, 0·81 division per second—an agreement within the limits of error of observation.

Electrification of its surface, due to contact with the medium in which it lives, must modify endosmotically the passage of substances into or out of a living cell; one might expect, therefore, that a part of the work of the cell would be expended in controlling this polarisation. It is unfortunately difficult to get reliable information on this point.

The fact that yeast and blood corpuscles migrate to the anode in isotonic sugar at different rates probably means that the negative charge per unit area on the red corpuscle is greater than that on the yeast cell, for, according to theory, the velocity, due to shedding of the charged fluid layer, is independent of the size or shape of a particle, provided the slip at the interface be small compared with the dimensions of the particle.* This last condition is usually held to be fulfilled by solid particles of finite size, but it must be remembered that the interface between the enormous molecules ot living matter and a fluid possibly differs widely in its properties from that between inert solid and fluid. Some features in the transport of fluid through living membranes seem to point to a very high coefficient of slip.

Another difficulty is that observation must be on cells in their natural habitat. Yeast and blood corpuscles in isotonic sugar solution are not in an indifferent medium, which leaves their properties unchanged. Isotonic sugar solution washes electrolytes out of muscle fibres, for instance, and so induces paralysis. The diffusion of salts out of yeast and blood corpuscles will polarise the surface to an extent determined by the osmotic properties of the surface and the nature of the salts.

The effect of poisons may be explained in this way. Chloroform, toluene, or traces of mercuric chloride reverse the sign of the charge on living cells, a second reversal, that is a return to the original charge, occurring after two or three days. The death change, however, is known to be accompanied by the liberation of salts, which previously were not " free,"† and the change in the polarisation of the surface may be referred to the diffusion of such salts out of the cell. The electrification of the surface certainly does not depend upon the intactness of the cell, for fragments of yeast cells broken up by pounding in a mortar moved in the same way, and at much the same rate, as did intact cells.

* Lamb, 'Brit. Assoc. Report,' 1887, p. 501.
† Macdonald, 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 322 ; Macallum, *ibid.*, 1906, B, vol. 77, p. 165.

In spite of this, we incline to the view that the surface charge does vary with variations in the state of activity of the living cell, for in a natural mixed culture of Gonium, Vorticella, and Amœba, the fact that different cells of *the same species* migrated at different rates was very noticeable. The observations were made in the water in which the cells had been living, exposed to air, so as to leave the respiratory exchange normal. Red blood corpuscles are living cells, with very slight or no intrinsic chemical activity. In correspondence with this, they were found to migrate in blood serum to the anode at a remarkably uniform rate.

Contact Potential at the Free Surface of Water.—When finely powdered graphite was sprinkled upon distilled water contained in the observation cell already described, and the current, led through non-polarisable electrodes, was not more than 0·002 ampère, the following phenomena were noticed:—Of the graphite particles some broke through the surface of the water and sank slowly, others floated unwetted; the latter therefore served as an index of the movements of the actual skin. Except near the upper and lower surfaces the graphite particles migrated to the anode, just *below* the free surface and just above the glass they migrated to the cathode. The unwetted floating particles either did not migrate at all, or performed relatively slow irregular movements, which were not reversed on reversing the direction of the current and were due to heating. The movements of the particles contained within the water were dead beat, and reversed with the current. We may take it (1) that the actual surface skin is not propelled at all, or so slowly that the movement escapes detection in a period of, say, five seconds, during which submerged particles immediately below have hurried half across the field of view; (2) that the layer of fluid immediately below is driven by the field past this skin in the same direction and with the same order of velocity as the water past the glass. If additional proof of this were wanted, it is to be found in the fact that yeast and red blood corpuscles move in opposite directions in the layer immediately below the free surface, just as they do in the layer next to the glass, and for the same reason, namely, because the more slowly migrating yeast cells are unable to stem the current of water.

The stationary layer is exceedingly thin. With oc. 4, ob. B, focussed on the floating graphite, submerged particles showing rapid movement are scarcely out of focus, and the spectacle produces a remarkable impression of the presence of a tenacious skin which has sufficient rigidity to act as a relatively fixed layer past which the subjacent water is being driven.

The flow of water in electric endosmose is due to " relatively enormous electric forces acting on the superficial film, and dragging the fluid (as it

were) by the skin through the tube."*   At the free surface of a fluid, therefore, there must be relatively† enormous forces dragging the surface skin and the water in opposite directions if the movement of the water be due to a difference of potential between it and a surface film of impurities condensed from the air or neighbouring solids. The only escape from this conclusion is that the movement of the water is due to a circulation produced by the endosmotic movement of the layer touching the glass, but any compensating circulation would be opposed in direction to the flow at the glass face, whereas the surface flow is in the same direction—it is, in fact, precisely what it would be if the air and surface film were replaced by a plate of glass. '

It seems difficult to avoid the conclusion that the film acts in the electric endosmose as though it were a rigid solid, and its properties are the same, when all ordinary precautions are taken to avoid contaminating the surface, as when a very thin layer of oil is allowed to spread over the water.

If the surface film really acts, as it would seem to, as a fixed layer past which the water is driven, since the stresses would be purely tangential, it is only necessary to regard it as having tenacity and as being anchored all round to the unwetted glass walls, and the apparent tenacity of the film will be partly true tenacity due to the forces between its component molecules and partly due to the work needed to rupture the film and expose a fresh water-air interface.

When the floating particles move at all, the movements are slight, irregular in direction (that is to say, they may be at an angle to the stream lines), and the direction is not reversed when the current is reversed. When the electrodes are placed directly in the distilled water, so as to cut out the large resistance of the end plates of porous earthenware, and the current thereby increased to 0·01 ampère or more, these movements are more rapid, and the submerged particles also now move in the same general direction as the floating particles, and their movement ceases to reverse when the electric field is reversed.

These movements, at first sight puzzling, admit of a very simple explanation. In the first place the direction is determined by the trough used and not by the current. That is to say, if the particles move from right to left no matter how the current is running, and the trough is displaced end for end, they now move from left to right. If we regard the gain of heat per unit of time from the current as being symmetrical with respect to the

* H. Lamb, *loc. cit.*

† Relative, that is, to the surface stresses in ordinary flowing due to differences of hydrostatic pressure.

electrodes, the observed effects would be produced by an unequal *loss* of heat at the two ends of the trough, due to the disposition of the materials, to differences in their specific heat, or to asymmetrical conductivity of heat. The result would be an unequal rise of temperature in the two halves of the chamber, and consequent differences in surface tension. If this explanation be correct, though the direction of the movement of submerged particles is independent of the direction of the current, the velocity past the observer should vary. This was found to be the case.

An analysis of the movements of the floating particles based on this hypothesis shows that in stronger fields the surface skin itself is dragged along. The following is an example :—Field approximately 35 volts per centimetre. Movement of floating particles always towards the right, but by reversal of current the velocity towards the cathode was 2,[*] towards anode 10. The drift due to heating therefore was 6, and the migration 4 divisions per second, and the latter was towards the anode. The surface film therefore was negative to the subjacent water.

<div align="center">APPENDIX, <i>July</i> 26, 1911.</div>

<div align="center"><i>The Electrification of Surface Films.</i></div>

<div align="center">By W. B. HARDY, F.R.S.</div>

The observations recorded in the preceding paper upon the endosmotic drift of the water in contact with a surface film involving foreign matter throw some light upon the range of molecular attraction. Under the conditions of the experiments, and for the short periods during which the current was on, it may be taken that there was no sensible hydrostatic pressure established due to change of level between the two ends of the trough. Under these conditions, if u be the velocity of the water past the anchored surface film, we have

$$u = \frac{d\phi}{dx}\,\sigma\frac{1}{\gamma},\text{†}$$

where σ is the electric density, and γ is a coefficient of sliding friction of water over the film.

The surface film acts as a thin sheet past which the fluid can flow, just as when the thickness of a soap film exceeds the range of molecular forces the interior mass may flow past the surface films which act as fixed boundary walls.

Whatever view be taken of the physical significance of the coefficient γ it must be related in some simple way to the forces of attraction of the water

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[*] Measured in divisions of the micrometer scale.

† H. Lamb, 'British Association Report,' 1887, p. 495.

for the superficial film.   So long as the depth of the effective film is greater than the range of the molecular forces the attractive forces across the inter-face will be constant for films of the same composition and at the same temperature.   When the thickness of the film is less than this range the Laplacian pressure at the interface, and therefore γ, must diminish and the velocity of the water under unit electric field increase.

The most probable assumption is that γ varies directly with the intrinsic pressure at the interface.

Let the attraction of a molecule of water upon a molecule of the film be $m m'\phi(f)$, where f is the distance between them.   Then, if z be the depth of the film and dz an infinitely thin plate, the attraction of the whole mass of the water on the film is

$$2\pi m \rho \int_z^\infty \pi(f) f\, df, \quad \text{where} \quad \pi(f) = \int_f^\infty \phi(f)\, df.$$

The density of the water may be taken as uniform.   The density of the film will vary rapidly.   Call its density ρ' and let

$$\psi(z) = \int_z^\infty \pi(f) f\, df.$$

The pressure at the interface will now be

$$2\pi\rho \int_0^z \rho'\psi(z)\, dz.$$

Leaving out of account for the moment the variation of density $\rho'(dz)$, and putting ρ equal to unity, we have

$$p = 2\pi \int_0^z \psi(z)\, dz, \quad \text{which is equal to} \quad 2\pi\left[z \int_z^\infty \pi(f) f\, df + \int_0^z \pi(f) f^2\, df\right].$$

Putting $\pi(f) = \mathrm{K}\beta^{-1}e^{-\beta f}$ as an analytically simple hypothesis, this integral reduces to

$$p = 2\mathrm{K}\pi\beta^{-4}\left[2 - e^{-\beta z}(z\beta + 2)\right],$$

where p is the pressure at the interface.

Rücker[*] gives as the range of molecular attraction $50\,\mu\mu$.   The estimate is based upon measurements of the thickness of soap films made by himself in association with Reinold, and upon a critical analysis of measurements by Quincke and others.   If β be put equal to 10^6 the force is approximately $\frac{1}{3}$ at $10\,\mu\mu$, $\frac{1}{150}$ at $50\,\mu\mu$, and vanishingly small at $100\,\mu\mu$.   Thus $\beta = 10^6$ approximates closely to Rücker's estimate.

[*] 'Journ. Chem. Soc.,' 1888, p. 222.

With this value, and on the assumption stated above, I find that the pressure at the interface would vary as follows:—

Depth of film, in $\mu\mu$.	Per cent. of maximal pressure.
50	95
40	90
30	80
20	66
10	45
5	25

The pressure would, however, not increase so rapidly as this with increase in the thickness of the film, owing to the variation of density in the film itself which is not taken account of in the above calculations. This is obvious when we remember that the film as it gains in thickness also gains in mean density owing to compression by the increase in the mean Laplacian pressure. The compressibility of the film will be relatively great since it is a transition layer between gas and fluid.

Turning to the observations themselves, the film was always so thin as to produce very slight effect upon the movements of shreds of camphor. This is what might be expected, since the distilled water was drawn from the bottom of a large glass reservoir, and all the chambers were thoroughly rinsed. From Rayleigh's measurements of such films* the thickness may be put with tolerable certainty as less than $2 \mu\mu$—probably $1.5 \mu\mu$.

At $2 \mu\mu$ the interfacial pressure will be considerably less than 10 per cent. of its maximal value, and the coefficient of sliding friction γ should have diminished proportionately.

Therefore, for the same potential gradient, the velocity of the water past the film should be much greater than it would be past a film $100 \mu\mu$ thick, or past the glass if we assume that the layer of electric density at the glass-water interface does not differ widely from that at the film-water interface.

So much for theory. Observation shows that the velocity of the water past the surface film differs very slightly from that past the glass at the bottom of the trough. Thus a surface film of a thickness far below the accepted estimate of the range of molecular action acts like a mass of solid of, relatively, infinite thickness.

In considering this surprising result the three variables on which the relative velocity at an interface depends have to be remembered. The

* 'Roy. Soc. Proc.,' 1890, vol. 47, p. 364.

external electric field being taken as the same in all cases, they are : (1) the electric density at the interface, (2) the coefficient of sliding friction (γ), and (3) variations in the attraction of water for different substances.

Taking these in the order mentioned, so far as I know it the literature of electric endosmose without exception supports the view that the electric density on surfaces in contact with water varies within narrow limits. The velocity of a submerged visible particle is independent of size and shape, and varies directly with the electric density on the particle. It was easy in our experiments to see chance fragments, motes of dust, and living cells, travelling with velocities which agreed to within 1 or 2 per cent. The evidence, therefore, is in favour of the view that the electric density at the film-water interface did not differ much from that at the glass-water face.

By hypothesis γ and $\phi(f)$, the coefficient of sliding friction and the intermolecular force, are dependent variables. If the thickness of the matter on each side of the interface exceeds the range of molecular attraction, β varies directly as $\phi(f)$, where $\phi(f)$ refers only to the molecular attraction across the interface.

Here, again, there is evidence that β does not vary. Putting the external electric field at unity, the velocity of a particle is given by the equation

$$V = -\sigma/\beta,^*$$

that is, in particles of 1μ diameter and upwards, the velocity is independent of size and shape. But if $\phi(f)$ and therefore β were different for different substances, the velocity should depend upon the nature of the particle.

Instead of this being the case we find protein masses, metals, and motes of dust in water, all moving in unit field with velocities of from 10 to 20×10^{-5} cm./sec., and the variations within this range can be traced to the influence of the chemical nature of the particle upon the polarisation of the interface.

We are thus driven to the conclusion that the adhesion of the film to the water practically reaches its maximum when the thickness is still much less than the accepted value for the range of the molecular forces.

In the case of a small sphere at a potential different from the water urged along by an electric field, the hypothesis which has been adopted would make γ sensibly constant until the diameter of the sphere fell to about $300\,\mu\mu$, when the pressure at the interface would be about 90 per cent.

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* Lamb, *loc. cit.*, p. 502.

of its maximal value.* This agrees with the fact that down to a diameter of 500 $\mu\mu$ the velocity still appears to be independent of size and shape.

It may be well, in conclusion, to emphasise the significance of the experiments. They seem to prove either that the coefficient of sliding friction between two phases is independent of the Laplacian pressure at the interface, or that the range of the molecular attraction is much less than Rücker's estimate—50 $\mu\mu$.

[P.S., *added July* 30.—During the present hot weather, when the water in the laboratory stands at 28° C., the film was found to have diminished in tenacity to a great extent. In order to give it the same degree of fixity under electrical stresses which it possessed at temperatures between 15° and 20°, it had to be thickened with oil until a blue film was produced, which almost entirely stopped the movements of camphor.]

The Origin of Osmotic Effects. IV.—*Note on the Differential Septa in Plants with reference to the Translocation of Nutritive Materials.*

By HENRY E. ARMSTRONG, F.R.S., and E. FRANKLAND ARMSTRONG.

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Our communication to the Society which was read on June 2 last year† was made under the primary title attached to the present communication, because it appeared to us that many of the osmotic phenomena in plants were to be correlated with effects produced initially by the class of substances to which we have ventured to extend the term *Hormone*, introduced by Starling but applied by him only to certain members of the group. The observations recorded were made with leaves of *Prunus laurocerasus.* In a

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* The integral for the pressure at the surface of a sphere in a vacuum is given by Rayleigh ('Phil. Mag.,' 1890 [2], vol. 30, p. 456), as $2\pi \int_0^{2r} f^2 \pi\left(f\right) df - \dfrac{\pi}{f} \int_0^{2r} f^3 \pi\left(f\right) df$.

Putting $\pi\left(f\right) = \dfrac{K}{\beta} e - \beta f$, this reduces to $\dfrac{\pi K}{\beta^2}\left[e^{-2r\beta}\left(4\dfrac{r}{\beta} + 8\dfrac{1}{\beta^2} + 6\dfrac{1}{r\beta^3}\right) - 6\dfrac{1}{r\beta^3} + 4\dfrac{1}{\beta^2}\right]$.

† "The Origin of Osmotic Effects. III.—The Function of Hormones in Stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures," 'Roy. Soc. Proc.,' B, 1910, vol. 82.